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Identification of Human and Animal Sources of *Escherichia coli* in South Carolina Estuaries

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ABSTRACT

Urbanization in coastal areas has caused increased point and nonpoint source runoff into estuaries. Fecal coliform bacteria levels are the current water quality indicators for shellfish harvesting areas. Harvesting areas are opened or closed based on the number of fecal coliforms present. This indicator is used to protect the public from pathogens associated with human waste, but all warm-blooded animals have fecal coliforms in the intestinal tract. A method to differentiate animal versus human sources of fecal coliform contamination could assist resource managers in developing strategies to protect shellfish harvesting areas. In this study, surface water samples were collected from both a developed and an undeveloped watershed in coastal South Carolina. Influent and effluent samples from several sewage treatment plants in the same area were also collected. Most Probable Numbers (MPNs) of fecal coliforms were determined for all samples. *Escherichia coli* from positive EC broth tubes were isolated on selective agar. These isolates were biotyped and confirmed as *E. coli* and analyzed for multiple antibiotic resistance (MAR) to 10 antibiotics. Then, MAR indices (# resistant / total # of antibiotics tested) x 100, were calculated for each isolate and site. Results indicated that MPNs from the developed watershed were significantly higher than those from the undeveloped watershed (p<0.05, Mann-Whitney Rank Sum). MAR analyses suggested there was a trend toward increased antibiotic resistance in samples from the urbanized watershed. In the Okatee River, three sites (20%) showed resistance to a single antibiotic (penicillin) suggesting nonpoint source runoff. In Broad Creek, five sites (47%) had antibiotic resistance, with the dominant resistance pattern to multiple antibiotics (chlortetracycline-oxytetracycline-tetracycline) suggesting a human source of contamination. Similarly, all raw sewage from treatment plants exhibited multiple antibiotic resistance. These results suggest that MAR testing may be a useful tool for differentiating *E. coli* from human and wildlife sources. Further testing of bacterial isolates from known animal sources is necessary to better assess the utility of this approach.

INTRODUCTION

Urbanization in coastal areas has caused increased point and nonpoint source runoff into estuaries. Almost one-third of South Carolina shellfish harvesting areas are closed because the consumption of oysters from these areas could pose a health risk to humans (Vernberg et al., 1992). Fecal coliform standards for shellfish harvesting are sometimes exceeded when no source of contamination can be identified (Kator and Rhodes, 1994).

Harvesting areas are opened or closed based on the number of fecal coliforms, mainly *E.coli*, and shoreline surveys for sources of fecal contamination. These indicators are used to protect the public from disease-causing microorganisms associated with human waste. All warm-blooded animals have fecal coliforms in the intestinal tract. Fecal coliforms quantified using traditional approaches may be due to both human and animal sources. Several researchers have investigated alternative water quality indicators. In a review by Kator and Rhodes (1994), proposed indicators of human pathogenicity include: other bacterial species more closely associated with humans, enzymes and whitening agents; but none of these methods to date have been proven better than the fecal coliform indicator.

A method to differentiate animal versus human sources of fecal coliform contamination could assist resource managers in developing strategies to more effectively manage shellfish harvesting areas. Multiple Antibiotic Resistance (MAR) is a method that could possibly be used to differentiate human from animal sources of *E. coli*. The rationale for this approach is that bacteria from human sources will be more resistant to antibiotics than bacteria from wild animals since humans receive antibiotic treatments, and wild animals do not. In previous studies this method has been shown to be useful in differentiating point from nonpoint pollution sources in Florida (Parveen et al., 1997) and in Maryland (Kaspar et al., 1990). This approach was applied in a field study of a developed estuary (Broad Creek), an undeveloped estuary (Okatee River) estuary and six sewage treatment plants (STP) in Beaufort County, South Carolina. See Figure 1.

Figure 1. Map indicating urban (Broad Creek) and reference (Okatee River) study areas in Beaufort County, South Carolina.

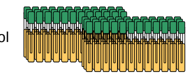
OBJECTIVES

- ♦Determine fecal coliform MPNs and isolate *E. coli* to measure MAR from various sources including humans, animals, sewage treatment plants, septic tanks and surface waters from pristine and urbanized areas.
- ♦Determine if urbanization increased the density and antibiotic resistance of *E. coli* in coastal waters.
- ♦Evaluate the utility of MAR in differentiating human from animal sources of *E. coli*.

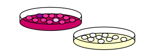
METHODS

Sample collection and preparation

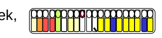
♦One sample from each site was collected in a sterile plastic jar in July, 1997.



♦Samples were analyzed at the South Carolina Department of Health and Environmental Control (DHEC) for fecal coliform numbers according to standard methods (APHA, 1992). Positive EC tubes were sent to the National Ocean Service (NOS), Charleston Lab for further analysis.

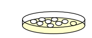


♦A total of 10 isolates from violet red bile agar plates were selected from each surface water sample and 15 isolates were chosen from each of the STP samples.

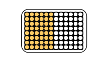


♦The identification of each isolate was confirmed as *E. coli* by API 20 E test kit (bioMerieux Vitek, Hazelwood, MO).

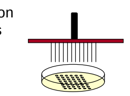
Multiple Antibiotic Resistance:



♦Confirmed *E. coli* isolates were further tested for MAR following the method of Parveen *et al.*, (1997), briefly described below.

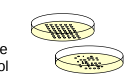


♦Isolates were transferred to a 96 well plate containing tryptic soy broth (TSB) and incubated for 4-6 h at 35 C.



♦The broth cultures were then transferred in duplicate with a 48-prong replicator to Mueller-Hinton agar plates, each containing one of 10 antibiotics, or to a control plate without antibiotics. Plates were incubated 18-24 hours at 35 C.

♦The antibiotics and their concentrations in agar were: ampicillin, 10 µl/ml; chlortetracycline, 25 µl/ml; kanamycin µl/ml, 25 µl/ml; nalidixic acid, 25 µl/ml; neomycin, 50 µl/ml; oxytetracycline, 50 µl/ml; penicillin G, 75U/ml; streptomycin, 12.5 µl/ml; sulfathiazole, 500 µl/ml and tetracycline, 25 µl/ml.



♦Resulting growth was measured and compared to the size of the same isolate on the control plate. **Resistance** = less than 15%reduction in colony size on the antibiotic plate compared to the control plate. **Sensitivity** = greater than or equal to 15% reduction in size compared to the control plate. Also calculated were:

♦**MAR index** (%) for an **isolate** = (# of antibiotics to which the isolate was resistant / total # of antibiotics tested) × 100.

♦**MAR index** (%) for a sample **site** = # of antibiotics to which all the isolates were resistant / (number of antibiotics tested × number of isolates per site) × 100.

RESULTS

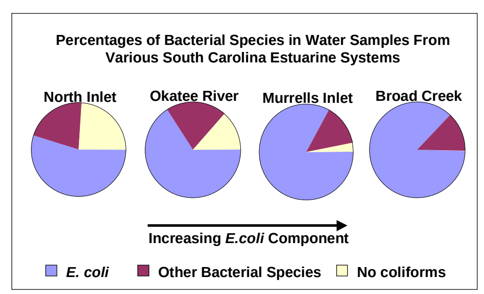


Figure 2. As an area becomes more developed the *E. coli* component increases. Note the similarities of bacterial species composition between the two undeveloped estuaries (North Inlet and Okatee River) and the two developed estuaries (Murrells Inlet and Broad Creek). Note: North inlet = NOAA NERRS reference site; Murrells Inlet = highly urbanized area near Myrtle Beach, South Carolina.

ANTIBIOTIC	SITE		
	Estuary Type		Sewage Treatment Plants, n=860
	Undeveloped (Okatie River), n=1061	Developed (Broad Creek), n=1443	
Ampicillin	0	4 (0.3%)	18 (2%)
Chlortetracycline	0	9 (0.6%)	6 (0.7%)
Kanamycin	0	1 (0.07%)	0
Naladixic Acid	0	1 (0.07%)	1 (0.1%)
Neomycin	0	0	0
Oxytetracycline	0	16 (1%)	9 (1%)
Penicillin G	10 (0.9%)	6 (0.4%)	46 (5%)
Streptomycin	0	0	8 (0.9%)
Sulfathiazole	1 (0.09%)	0	6 (0.7%)
Tetracycline	0	12 (0.8%)	12 (1%)
Number of Sensitive Isolates	1050 (99%)	1394 (97%)	754 (88%)
Number of Resistant Isolates	11 (1%)	49 (3%)	106 (12%)
Overall MAR (%)	1.04	3.40	12.33

Table 1. Summary of Antibiotic Resistance Among the Sites Tested. In Broad Creek, 49 of 1443 (3%) isolates were resistant to antibiotics. The dominant MAR pattern was Chlortetracycline-Oxytetracycline-Tetracycline. In the Okatee River, 11 of 1051 (1%) isolates were resistant to antibiotics. All isolates from the Okatee, except one, were resistant to only penicillin. At STPs the dominant MARs were ampicillin, penicillin and the tetracyclines.

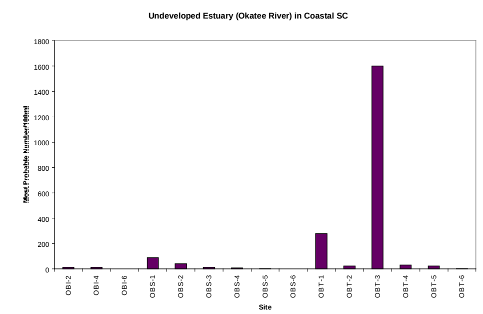


Figure 3. In the Okatee River, eight out of 15 (53%) sites met the fecal coliform standard for approved shellfish harvesting waters. The one site that had an MPN of 1600 was directly adjacent to a sewage treatment plant drain field.

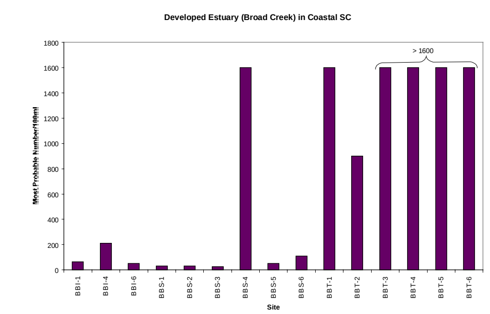


Figure 4. MPNs from the developed, Broad Creek watershed, were significantly higher than those from the undeveloped watershed (Mann-Whitney, p<0.05). None of the stations met the fecal coliform standard for approved shellfish harvesting waters.

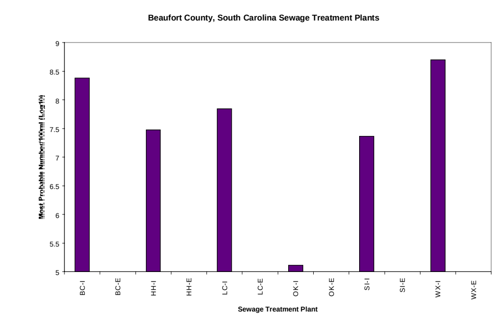


Figure 5. MPNs in raw sewage from STPs were significantly higher than MPNs of the surface water samples, in both developed and undeveloped estuaries (Mann-Whitney, p<0.05). All treated sewage effluent samples had MPNs <2.

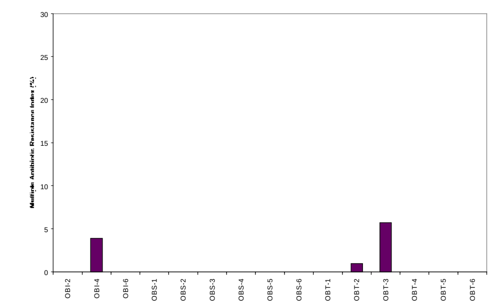


Figure 6. In the Okatee River, three out of 15 (20%) sites showed antibiotic resistance.

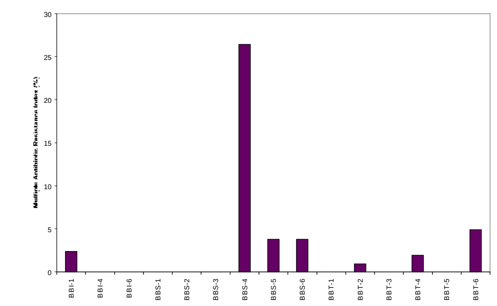


Figure 7. In Broad Creek, seven of 15 (47%) sites showed antibiotic resistance.

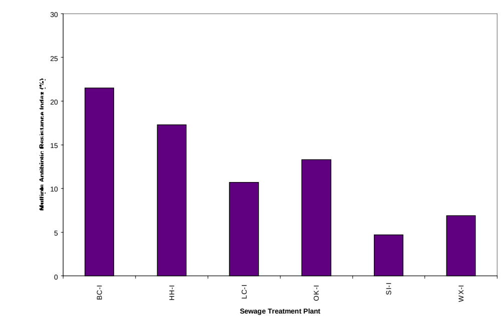


Figure 8. All of the sewage treatment plant samples contained bacteria with antibiotic resistance. Of 87 isolates, 21 (24%) were resistant to multiple antibiotics.

Okatee River: Coliform Bacteria and Multiple Antibiotic Resistance Results

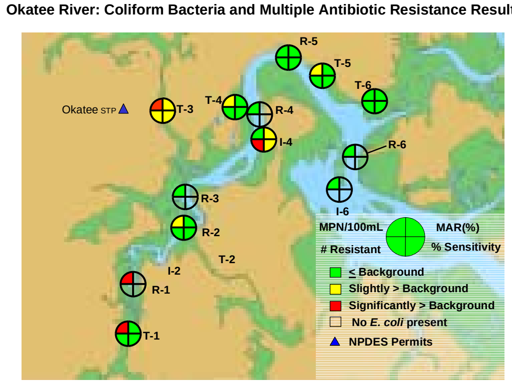


Figure 9. In the Okatee River, eight out of 15 (53%) sites were below background levels for MPNs, and 12 out of 15 (80%) sites were below background levels for MARs. The site with the highest antibiotic resistance was directly adjacent to a sewage treatment plant spray field, and a second site with MAR (penicillin and sulfathiazole) was located just down stream. ▲ Sewage Treatment Plants: Okatee = OK.

Sites labeled as R on these maps are synonymous with sites ending in S on the above graphs.

Key for Figures 9 and 10

MPNs: ≤13 (Background=BG); >13≤43 (Slightly>BG); >43 (Significantly>BG)

MAR: 0 (BG); >0≤12.3 (Slightly > BG); >12.3 (Significantly > BG)

 # Resistant: 0 AB (BG); 1-3 AB (Slightly > BG); >3 AB (Significantly > BG) (# Antibiotics = AB)

% Sensitivity: 100% (BG); 85-99% (Slightly > BG); <85% (Significantly > BG)

Broad Creek: Coliform Bacteria and Multiple Antibiotic Resistance Results

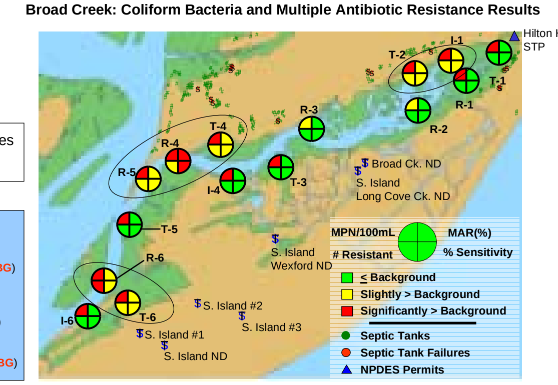


Figure 10. Overall, MPNs and antibiotic resistance for Broad Creek were higher than the Okatee River. Note the three regions with antibiotic resistance, two areas with failing septic tanks and one area near NPDES permitted sites. See circled sites. ▲ Sewage Treatment Plants: Broad Creek ND = BC, Hilton Head PSD = HH, Long Cove Creek ND = LC, South Island ND = SI, Wexford ND = WX.

CONCLUSIONS

♦MPNs were statistically significantly higher in the developed estuary than in the undeveloped estuary.

♦Percentage of *E. coli* in the overall fecal coliform composition increased with increasing development.

♦*E. coli* antibiotic resistance was higher in the urbanized estuary than in the pristine estuary.

♦The sewage treatment plant MPNs and MARs were statistically significantly higher than the MPNs and MARs of either estuary.

♦Significant spatial correlation was found with MPN/MAR results and known human pollution sources in both estuaries.

♦MAR is a useful tool for differentiating point source from non-point source fecal pollution.

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REFERENCES

Kaspar, C. W., J. L. Burgess, I. T. Knight, and R. R. Colwell. 1990. Antibiotic resistance indexingof *Escherichia coli* to identify sources of fecal contamination in water. Can. J. Microbiol. 36:891-894.

Kator and Rhodes. 1994. Chapter 2: Microbial and Chemical Indicators. In **Environmental Indicators and Shellfish Safety**. Eds. C. R. Hackney and M. D. Pierson. Chapman and Hall, New York. pp. 30-91.

Parveen, S., R. L. Murphree, L. Edmiston, C. W. Kaspar, K. M. Portier, and M. Tamplin. 1997. Association of multiple-antibiotic-resistance profiles with point and non-point sources of *Escherichia coli* in Apalachicola Bay. Appl. Environ. Microbiol. 63:2607-2612.

Vernberg, F.J., W.B., E.R. Blood, A.R. Fortner, M.H. Fulton, H. McKellar, K.W. Michener, G.I. Scott, T.C. Siewicki and K. El-Figi. 1992. Urbanization and Southeastern Estuarine Systems (USES): Research Proposal and Progress Report. 299 pages.